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Fossil evidence of proboscidean frugivory and its lasting impact on South American ecosystems

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Most megaherbivores in the Americas went extinct around 10,000 years ago, presumably disrupting the long-distance seed dispersal of large, fleshy-fruited plant species. The neotropical anachronism hypothesis, proposed by Janzen and Martin, suggests that large fruits evolved in response to past selective pressures from now-extinct megafauna. While this explains many key adaptations of 'megafaunal fruit' plants, it lacks robust palaeoecological evidence. Here we use a multiproxy approach that provides evidence of frugivory by the extinct South American proboscidean *Notiomastodon platensis*. Using a machine learning model that reveals the extinction effects of these megafaunal dispersers in modern ecosystems, we show how losing seed dispersal interactions increased the extinction risk of megafaunal fruit plants in regions across South America lacking smaller surrogate dispersers. Our results imply that current ecosystems may undergo further functional changes under continuing anthropogenic pressures.

Approximately half of the world's large-bodied mammal species went extinct at the end of the Late Pleistocene, triggering new and enduring ecological states[1]. Extinctions were particularly dramatic in South America, with 80% of large mammals (more than 44 kg) and all mega mammals (more than 1 t) disappearing[2]. These losses had far-reaching effects on ecological interactions and networks, triggering cascading effects on the community structure and stability[3–5]. One key disrupted function was the long-distance seed dispersal (LDSD) of plant species with fruits presumably adapted to megafaunal dispersal[6–8]. According to Janzen and Martin[7], these plants feature overbuilt, fleshy fruits that lack effective present-day seed dispersers, constituting neotropical seed dispersal anachronisms (the Janzen and Martin neotropical anachronisms, or JMNA, hypothesis).

The JMNA hypothesis predicts that the loss of LDSD provided by the extinct megafauna should have led to declines in the distribution and genetic diversity of many plant species, potentially increasing their likelihood of extinction[9]. Two fundamental assumptions underlie this prediction. First, the extinct megafauna regularly included large fruits in their diet and, second, their dispersal services were effective. Consequently, their extinction would have severely handicapped early plant establishment, resulting in a higher extinction risk for those plants with a megafaunal seed dispersal syndrome. Several recent theoretical

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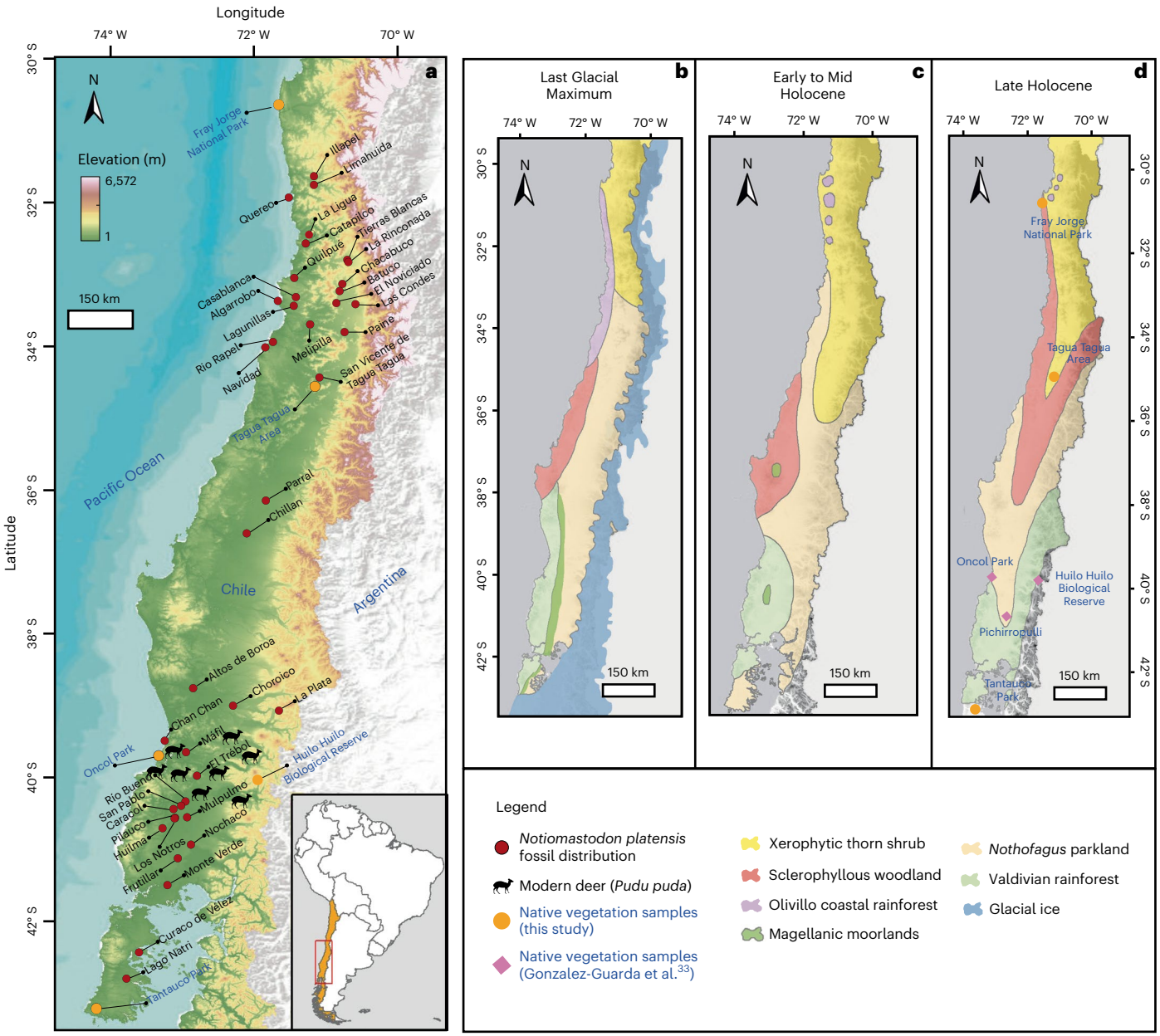

Fig. 1 | Geographical setting of the samples studied from central Chile. **a**, Geographical distribution of *N. platensis* in central Chile, marked by the locations where fossilized molars were unearthed. This map also highlights the areas from which both modern vegetation samples and contemporary deer specimens (*P. puda*) were collected to provide calibration for the *N. platensis* analyses. **b**–**d**, Sequential stages of landscape evolution during the Late Pleistocene: Last Glacial Maximum (**b**), Early to Mid Holoene (**c**) and Late Holocene (**d**). This temporal progression offers insights into the environmental changes that shaped the habitat of *N. platensis*.

models support these predictions[10–13], with some suggesting that no living non-human mammal species have replaced gomphotheres (extinct proboscideans) as primary seed dispersers in South America[8]. Empirical studies on extant species have further documented the impacts of functional extinctions in defaunated landscapes[14–17], including shifts in the phenotypic evolution of some plant species[18]. Genetic variation generally declines with shrinking population sizes[19], yet some studies on megafaunal fruit plants have found no genetic evidence to support the loss of LDSD[20]. Those studies have suggested that seed dispersal by smaller, extant vertebrates may have partially mitigated the effects of the megafaunal extinctions. Given the mixed evidence surrounding the JMNA hypothesis, integrating palaeoecology with studies of current ecosystems could offer deeper insights into the lasting ecological impacts of megafaunal loss.

A 'smoking gun' for the JMNA hypothesis would require indisputable evidence of frugivory by the extinct megafauna, providing strong support for the link between the extinction risk of megafaunal fruit plants and the loss of LDSD. Such evidence must go beyond demonstrating their consumption of a single food type and should include ecosystem reconstructions to assess whether frugivory was a recurring ecological interaction that aligned with the local palaeoenvironment[21]. For example, environments with a predominance of tree biomass could indicate higher fruit consumption[4]. Direct evidence of frugivory in the Americas remains limited, although the dung[22–24] and gut contents of mastodons in North America[25], as well as the dental calculus[26] and analysis of the dental microwear of Brazilian gomphotherids provide some clues. However, recent studies on the dental microwear and dental calculus of North American proboscideans have revealed no signs of fruit consumption[27,28].

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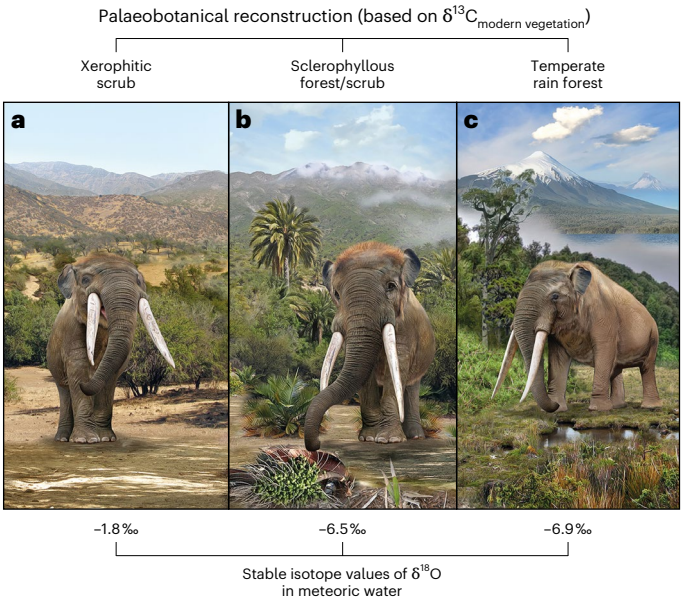

Fig. 2 | Palaeoenvironmental reconstructions of the landscapes occupied by *N. platensis* in central Chile. a, Xerophytic vegetation. **b**, Sclerophyllous vegetation. **c**, Valdivian and northern Patagonian forest vegetation. We used stable isotope analyses of modern vegetation and fauna (the deer *P. puda*), integrated with our analyses of *N. platensis* remains, to infer these individual scenarios.

Using a multiproxy approach, we sought robust evidence of frugivory in the remains of an extinct American megafaunal mammal—*Notiomastodon platensis* from central Chile (Fig. 1 and its accompanying source data)—and explored the palaeoenvironmental conditions that may have supported this behaviour. *Notiomastodon platensis* was a very large (4.4 t or larger) and widespread South American proboscidean that became extinct at the end of the Pleistocene[29]. Although primarily a leaf-eater[30–33], *N. platensis* likely consumed fruits and acted as a seed disperser. To interpret its diet, we first reconstructed the feeding environment of *N. platensis* by integrating stable isotope data from fossil molars, modern mammals and modern vegetation. Second, we examined fossil molars from specimens found across an approximately 1,500-km range in Chile and used dental calculus, dental microwear and stable isotope analyses to determine the types of plant material these animals consumed. Finally, we assessed how the extinction risk differed between megafaunal and non-megafaunal fruit plants in a defaunated neotropical ecosystem compared to ecosystems where smaller-sized dispersers (for example, primates, agoutis and tapirs) were still present. We predicted that megafaunal plants would face higher extinction risks in a defaunated ecosystem than in ecosystems where smaller-sized vertebrates may still act as surrogate dispersers.

The foraging palaeoenvironment of *N. platensis*

To infer the palaeoenvironment of *Notiomastodon platensis* in Chile, we estimated the proportional contributions of C_3 and C_4 plants to its diet (piC_3 and piC_4), as well as its standardized isotopic niche width (B_A). Low carbon isotope ($\delta^{13}C$) values in the bioapatite of *N. platensis* specimens suggest a specialized diet in C_3-dominated environments in central Chile. Specimens from south-central Chile (38–42° S) exhibited the most negative $\delta^{13}C$ values, indicating a leaf browser adapted to closed forests environments ($B_A = 0$; $piC_3 = 1$) (Supplementary Table 1). By contrast, specimens from north-central Chile (31° S–36° S) with high $\delta^{13}C$ values suggest a slightly mixed C_3/C_4 diet ($B_A = 0.16$; $piC_4 = 0.07$; $piC_3 = 0.93$). from a mosaic and season-dependent open landscape. On average, *N. platensis*'s diet contained between around 7% and ~2% of C_4 plants (Supplementary Table 1). The palaeoenvironmental stable isotope ranges developed by Domingo et al.[30] show that *N. platensis* probably inhabited woodland–mesic C_3 grassland environments (−14.5 to −9.5‰) in both regions. Lower $\delta^{13}C$ values (−13.3 ± 0.8‰) from samples from south-central Chile suggest a forested environment (Fig. 2 and the accompanying source data).

A comparison of our data with data available on the current vegetation of north-central Chile (considering the current $^{13}C_{atmCO2}$ composition of −8‰) indicates that *N. platensis* fed in either sclerophyllous forests or sclerophyllous scrub ($\delta^{13}C_{bioapatite} = -12 \pm 1‰$; $\delta^{13}C_{estimated\ consumed\ plants} = -27 \pm 1‰$), as well as in xerophytic scrub ($\delta^{13}C_{bioapatite} = -7 \pm 2‰$; $\delta^{13}C_{estimated\ consumed\ plants} = -22 \pm 2‰$). The *N. platensis* specimens from south-central Chile ($\delta^{13}C_{estimated\ consumed\ plants} = -28.3 \pm 0.8‰$) probably inhabited more-humid and closed forests, including the Fray Jorge National Park hydrophilic forest ($\delta^{13}C_{modern\ vegetation} = -30.3 \pm 2‰$) or hydrophilic and sclerophyllous forests, such as those found at Tagua Tagua ($\delta^{13}C_{modern\ vegetation} = -29 \pm 2‰$) (Supplementary Table 2).

By comparing the stable isotope values of modern vegetation ($\delta^{13}C_{modern\ vegetation} = -31 \pm 2‰$ from this study and $\delta^{13}C_{modern\ vegetation} = -33 \pm 2‰$ from González-Guarda et al.[33]) with the corrected $\delta^{13}C_{bioapatite}$ values (−27 ± 2‰ for north-central Chile and −28.3 ± 2‰ for south-central Chile, applying a +15‰ correction factor), it appears that *N. platensis* inhabited more-open forests than those found in the present day (Fig. 2). A comparison of $\delta^{13}C_{bioapatite}$ values from South American gomphotheres (that is, Ecuador, Perú, Bolivia, Chile, Argentina and Brazil), reveals significant differences among localities ($X^2 = 160.08$, d.f. = 6, $P < 0.001$). Specifically, we identified two localities with very different vegetation (Dunn's post hoc test with Bonferroni correction, $P < 0.05$) (source data for Fig. 3). Among all regions, gomphotheres from north-central and south-central Chile show the strongest tendency to inhabit forested environments (Fig. 3).

Meteoric water oxygen isotope ($\delta^{18}O$) values derived from *N. platensis* molars (Extended Data Fig. 1 and Supplementary Table 3) were calibrated using $\delta^{18}O_{bioapatite}$ values obtained from the extant deer *Pudu puda* ($n = 8$; −6.6 ± 1.5‰) and suggest a climate akin to that of central Chile today (present range: around −3 to −10‰). The average $\delta^{18}O_{bioapatite}$ values from *N. platensis* in north-central and south-central Chile are −6.5 ± 2‰ ($n = 54$) and −6.9 ± 1‰ ($n = 31$), respectively. A smaller subset of north-central samples ($n = 10$) indicates very warm conditions (−1.8 ± 1‰), whereas only two samples (La Rinconada, 33° S, Tagua Tagua, 34° S) suggest a colder, glacial environment (−11.76 and −14.98‰, respectively). This implies that gomphotheres probably foraged in warmer environments with interglacial or interstadial climates (Fig. 2). An analysis of the $\delta^{18}O$ values from South American gomphotheres (that is, Ecuador, Perú, Bolivia, Chile, Argentina and Brazil) shows statistically significant differences among the different localities ($F_{6,29.2} = 52.5$, $P < 0.001$), specifically, between Argentina and north-central Chile ($P < 0.001$), Brazil and north-central Chile ($P < 0.001$) and Argentina and south-central Chile ($P < 0.001$) (pairwise *t*-tests with Bonferroni correction) (Fig. 3 and the accompanying source data). To better understand these differences, future studies should establish modern meteoric water baselines for each region. This approach could help interpret the factors influencing the $\delta^{18}O$ values (for example, altitudinal and latitudinal effects).

Palaeontological evidence for long-term frugivory

Two independent lines of evidence suggest that *N. platensis* had a frugivorous diet. Indirect evidence comes from stable isotope analyses, whereas direct evidence is provided by dental calculus and dental microwear analyses (Supplementary Tables 4–6). The $\delta^{13}C_{bioapatite}$ values indicate that 80% of *N. platensis* specimens exhibited behaviours primarily indicating browsing in wooded environments (Fig. 2), implying incidental or voluntary fruit consumption. Frugivory, as interpreted from the dental calculus and dental microwear analyses, was evidenced in diverse ecosystems ranging from dense temperate C_3 forests (for example, La Plata, 40° S) to more-open C_3/C_4 vegetation (for example, Tagua Tagua, 34° S), but was more common in north-central Chile

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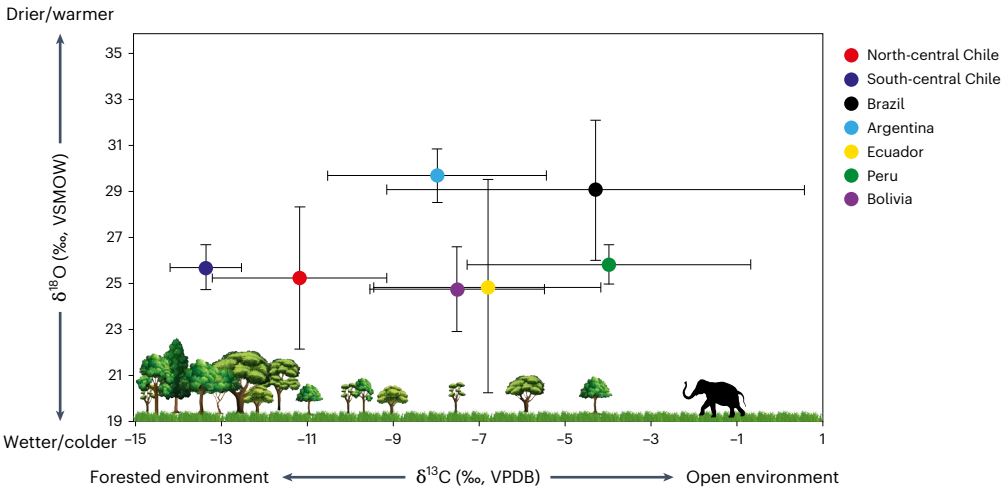

Fig. 3 | Stable isotope values ($\delta^{13}C$ and $\delta^{18}O$) from South American gomphotheres. Bioapatite mean $\delta^{13}C$ (‰, VPDB) and $\delta^{18}O_{CO3}$ (‰, VSMOW) values ±1 s.d. (from bones and molars of the gomphotheres *N. platensis* and *Cuvieronius hyodon* from South America). Both unpublished samples and previously published samples were used (see section *N. platensis* and *C. hyodon* (South America) in Supplementary Data). The $\delta^{13}C$ values from the different localities showed statistically significant differences (Kruskal–Wallis, $X^2 = 160.08$, d.f. = 6, $P < 2.2 \times 10^{-16}$). The $\delta^{18}O$ values from the different localities showed statistically significant differences (analysis of variance, $F_{6,29.2} = 52.5$, $P < 0.001$). The average $\delta^{13}C$ and $\delta^{18}O_{CO3}$ values of gomphotheres from different South American regions are as follows: north-central Chile ($n = 93$, $\delta^{13}C = -11.1 \pm 2‰$), ($n = 93$, $\delta^{18}O_{CO3} = 26.3 \pm 3.09‰$); south-central Chile ($n = 31$, $\delta^{13}C = -13.3 \pm 0.8‰$), ($n = 31$, $\delta^{18}O_{CO3} = 25.7 \pm 0.98‰$); Brazil ($n = 83$, $\delta^{13}C = -4.2 \pm 4.8‰$), ($n = 79$, $\delta^{18}O_{CO3} = 29.08 \pm 3.07‰$); Argentina ($n = 43$, $\delta^{13}C = -7.9 \pm 2.5‰$), ($n = 43$, $\delta^{18}O_{CO3} = 29.8 \pm 1.19‰$); Ecuador ($n = 16$, $\delta^{13}C = -6.75 \pm 2.6‰$), ($n = 15$, $\delta^{18}O_{CO3} = 24.9 \pm 4.5‰$); Peru ($n = 4$, $\delta^{13}C = -3.8 \pm 3.6‰$), ($n = 4$, $\delta^{18}O_{CO3} = 25.3 \pm 1‰$); and Bolivia ($n = 8$, $\delta^{13}C = -7.22 \pm 2‰$), ($n = 8$, $\delta^{18}O_{CO3} = 25.2 \pm 1.5‰$). See the 'gomphotheres *P* value' section in Supplementary Data for more details about the statistics.

(31–36° S), where a C_3 diet dominated (Fig. 2). The dental calculus and microwear analyses point to a fruit-based diet. Specifically, 27 out of 60 dental calculus samples from *N. platensis* molars contained clear markers of fruit consumption, such as starch, sclereids and raphides (Supplementary Table 4). The starches included both common reserve starches and rarer, transitory starches[34–36] (Fig. 4a). Although many of the starch granules were unidentifiable and not all granules were diagnostic[37], polyhedral starch grains (Fig. 4c) suggested the consumption of Poaceae[38] seeds, including *Chusquea* sp. (Fig. 4h), a common bamboo of central-southern Chile. Additionally, we found ellipsoidal starches from legumes[30] in molar 47b (Fig. 4b). Raphides in molars PV15 and MHNV1 (Fig. 4i) point to the consumption of seeds of the Arecaceae (palm) family[39] and, in particular, to the fruit of *Jubaea chilensis*, the only native Chilean palm[40]. Other palm indicators include tracheary elements, psilate and alternate echinate spheroid morphotypes (Fig. 4e–g)[41,42]. *Jubaea chilensis* nuts lack phytoliths and, thus, gomphotheres probably consumed ligneous fruit parts, including sclereids. Molars PV235 and LP15 exhibited brachysclereids (Fig. 4k), typically found in fruit pulp, but also stems. Macrosclereids were observed in PV235 (Fig. 4j), and osteosclereids (Fig. 4l)—bone-like cells found in seed tests and fruit walls—were present in PV264, PV47B, PV47F and PI14. Although brachysclereids alone may not provide conclusive evidence of fruit consumption, their presence alongside reserve starches in samples PV235 and LP15 further supports a frugivorous diet.

Tooth microwear patterns from central Chile reveal that *N. platensis* predominantly browsed on leaves, as evidenced by a high number of pits (21.7) and a relatively low number of scratches (13.9) (Fig. 5 and the accompanying source data, and Supplementary Table 6). Modern Elephantidae exhibit similar patterns (Supplementary Table 6), with the forest elephant (*Loxodonta cyclotis*) exhibiting the most pits (29.8) and the Asian elephant (*Elephas maximus*) showing the most scratches (18.3) (Supplementary Table 6). However, *N. platensis* differs from these extant species in having a much higher proportion of puncture pits (89.4%) (Supplementary Table 6) and gouges (76.3%). Additionally, *N. platensis* molars mainly feature coarse scratches (scratch width score (SWS) = 2), whereas its living relatives display more hyper-coarse scratches (*Loxodonta africana* SWS = 2.8; *L. cyclotis* SWS = 3.1; *E. maximus* SWS = 3.1). A high occurrence of puncture pits is typically associated with fruit and hard seed consumption because these foods contain phytoliths. Fruit rinds in some dicotyledonous plants produce large phytoliths or a high number of phytoliths that are related to the presence of puncture pits in large, fruit-browsing mammals[43,44]. This dental evidence distinguishes *N. platensis* from other leaf-browsing herbivores and suggests a closer link to fruit and seed ingestion[11,45].

The extinction risk of neotropical trees

Our machine learning model reveals that a significant percentage of modern megafaunal fruiting plants are at increased risk of extinction when seed dispersers are unavailable (Fig. 6). In central Chile, 40% of megafaunal fruit-bearing plants (Fig. 6) are threatened to some degree (that is, Critically Endangered (CR), Endangered (EN) or Vulnerable (VU) categories of The International Union for Conservation of Nature (IUCN), a rate four times higher than that of non-megafaunal fruit plants in the same area (10%). This rate is also disproportionately higher than the percentage of threatened megafaunal (range: 4–10%) and non-megafaunal (range: 5–18%) fruit plants in three other neotropical regions[46] where medium-sized frugivorous mammals still exist (Fig. 6). Our null model, which randomized the conservation statuses of species across groups by site and fruit type, further suggests that megafaunal species in central Chile are more likely to be threatened with extinction (40%) than would be expected by chance (with the null hypothesis rejected, the 95% randomized values ranging from 0 to 21.4%). Consequently, trees bearing megafaunal fruits in the only region lacking surrogate dispersers in modern times face a higher extinction risk compared to those in regions where dispersal interactions have not been completely disrupted.

Discussion and conclusions

Our research presents unequivocal and robust fossil evidence of fruit-eating behaviour in an extinct proboscidean (Figs. 4 and 5 and Supplementary Video). Furthermore, our machine learning model suggests that the loss of dispersal services increases the extinction risk for megafaunal fruit plants, particularly in regions like southern South

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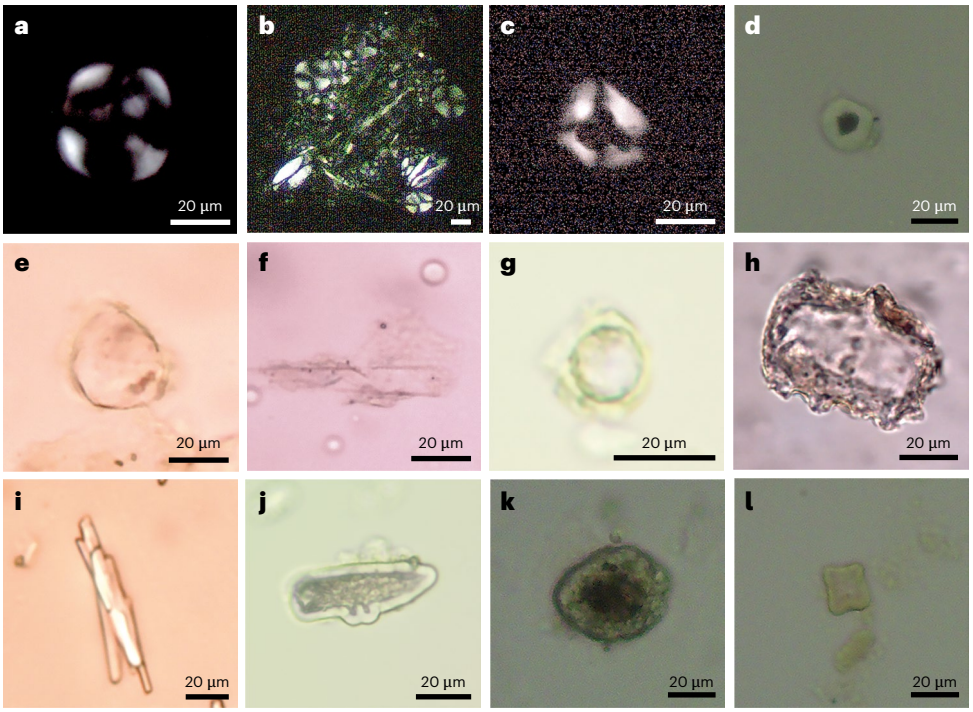

Fig. 4 | Microfossils from the dental calculus. a–**d**, Reserve starches: globular (**a**), ellipsoidal aggregate (**b**), polyhedral (**c**) and starch with birefringence loss (**d**). **e**–**g**, Phytoliths: smooth spheroid (**e**), tracheid (Arecaceae type) (**f**) and altered spheroid echinate (**g**). **h**, Bulliform (*Chusquea* sp. type). **i**, Raphides. **j**–**l**, Sclereids: macrosclereid (**j**), brachysclereid (**k**) and osteosclereid (**l**). The concentration of microfossils in the samples was calculated independently on three slides by counting *Lycopodium* spores, which were added as an external marker.

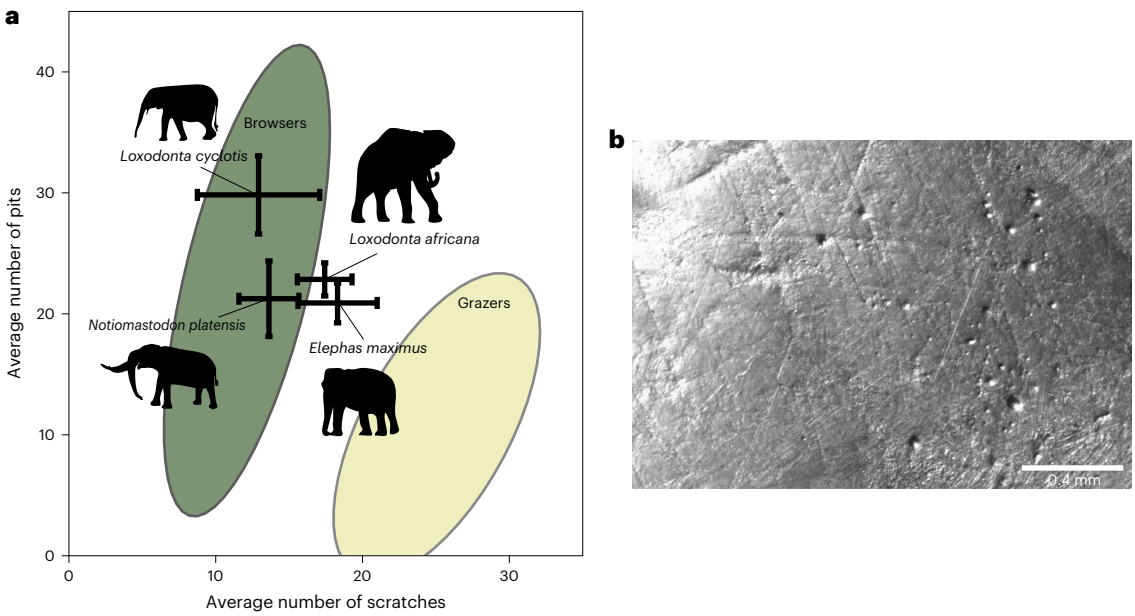

Fig. 5 | Bivariate plots of the average numbers of pits and scratches of molars from modern proboscideans and *N. platensis*. a, The molars from central Chile were compared to those of the three extant Elephantidae (data from Rivals et al.[76]; Semprebon et al.[77]). Error bars represent ±1 s.d. Ellipses represent 95% confidence intervals for extant ungulate browsers and grazers[37]. **b**, Photomicrograph of the tooth enamel surface of a selected specimen of *N. platensis* at ×35 magnification. Several puncture pits are visible.

America where medium-sized vertebrates capable of acting as surrogate dispersers are absent (sensu Guimaraes et al.[6]) (Fig. 6). These findings support the JMNA hypothesis[6,7] and underscore the lasting ecological consequences of losing large frugivores from modern ecosystems.

The role of *N. platensis* as a seed disperser has been debated due to the lack of multiple lines of evidence that, when integrated, would confirm its frugivory. Our stable isotope analysis offers insights into the palaeoenvironment of *N. platensis*, revealing that these animals foraged in areas with large standing biomasses of shrubs and/or trees, as indicated by low $\delta^{13}C$ values (Fig. 2). While frugivory cannot be directly inferred from stable isotopes, the predominance of tree biomass in the Late Pleistocene of central Chile (Supplementary Discussion) suggests at least occasional fruit consumption. The consumption of leaves and branches was also important because these are available year-round

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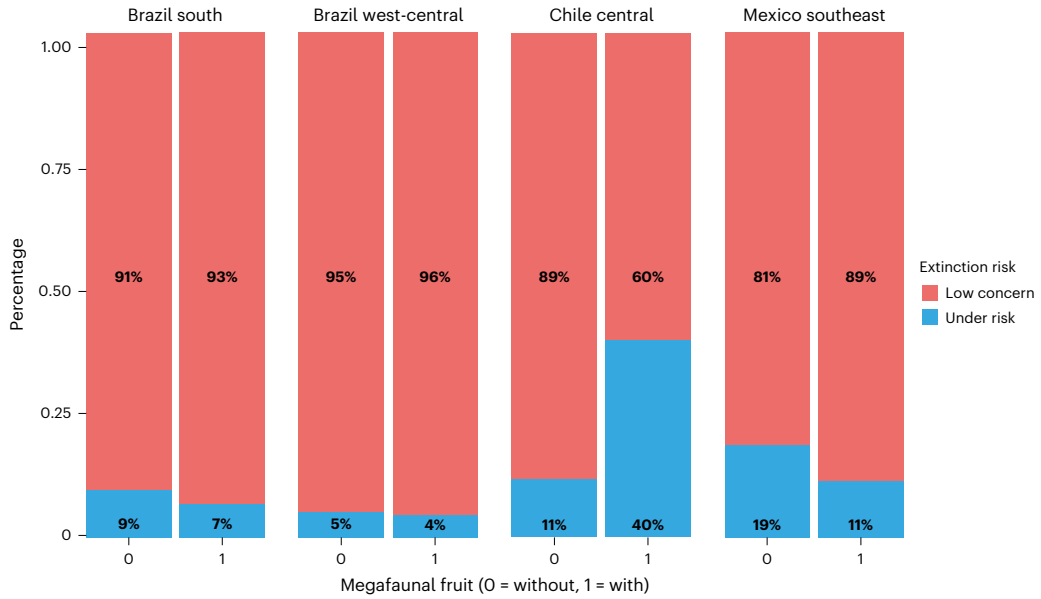

Fig. 6 | Percentage of tree species in two extinction risk categories. Each bar differentiates between tree species with (1) and without (0) megafaunal fruits in four botanical regions in South America: Brazil south, Brazil west-central, Chile central and Mexico southeast. The conservation categories Low concern, covering the Near Threatened (NT) and Least Concern (LC) categories, and Under risk, covering the Vulnerable (VU), Endangered (EN) and Critically Endangered (CR) categories, are as defined by the IUCN Red List of Threatened Species, with missing data imputed using a machine learning classification algorithm. Fruit types were assigned to each species based on an extensive literature review.

and provide more protein than fruit pulp[47]. However, during the fruiting season, calorie-rich fruits may have been a preferred resource, similarly to the behaviour of modern forest elephants[48], which have specialized olfactory adaptations to detect fruits[49].

Our $\delta^{18}O$ data (Extended Data Fig. 1 and Supplementary Table 3), combined with modern plant $\delta^{13}C$ values, suggest that gomphotheres in central-northern Chile inhabited Mediterranean-like environments characterized by dry summers and humid winters. These conditions would have been favourable for the growth of fleshy-fruited plants during the Late Pleistocene[32] (Supplementary Discussion), a hypothesis supported by recent evidence for the presence of Mediterranean ecosystems during this period[50]. During the fruiting season—central Chile's hot and dry months—populations of *N. platensis* from the south-central region may have migrated to these areas to meet their nutritional needs. This behaviour would parallel that of modern elephants, which track fruit sources throughout their lives, often creating permanent trails to ensure consistent access[51] (Supplementary Table 7).

Our findings support the documented generalist feeding behaviour of *N. platensis* in South America[46,52], its diet shaped primarily by the dominant flora in its environment[28]. *Notiomastodon platensis* occupied a wide range of habitats, from open C_4 environments at equatorial latitudes to cold, rainy forests in northern Patagonia, Chile (Fig. 6)[32]. As with modern elephants, the diversity of ecosystems they inhabited probably resulted in regional differences in their fruit consumption. This is evidenced by their presence in both open environments, where frugivory was limited, and in the forested regions of Brazil (that is, $\delta^{13}C = -15.4 \pm 1.6‰$)[53], such as along the Juruá and Madeira rivers in the states of Acre and Rondonia, where fruit probably played a more prominent role in their diet (source data for Fig. 3).

Our dental calculus and dental microwear analyses further confirm that the *N. platensis* in central Chile included fruits in its diet. This evidence not only establishes the varied diet of *N. platensis*, but also highlights its potential role as a seed disperser, a function previously inferred primarily from speculative morphological assessments. Although seed dispersal by extinct mammals is believed to have been critical for the LDSD of megafaunal fruit plants[8], the full impact of the loss of these frugivores on present-day plant communities remains unclear. According to the JMNA hypothesis[7], the extinction of large dispersers should have led to significant declines in the recruitment success of megafaunal plants. Without these dispersers, megafaunal plants potentially experienced sharp reductions in their geographical range sizes and genetic diversity, thereby increasing their risk of extinction[4,18,54].

Three lines of evidence provide strong support for this hypothesis. First, large fleshy-fruited plant species, such as *J. chilensis* (Areaceae), *Beilschmiedia miersii* (Lauraceae), *Geoffroea decorticans* (Fabaceae), *Gomortega keule* (Gomortegaceae) and *Trichocereus chilensis* (Cactaceae) (Extended Data Fig. 2), which once thrived in diverse South American environments, appear to have experienced significant range contractions during the Early Holocene. This fragmentation led to discontinuous populations (Extended Data Fig. 2). Genetic studies further reveal substantial diversity loss, probably driven by disrupted seed dispersal after the Pleistocene. Some studies have suggested that humans in South America expanded or maintained the distribution of many fruit species that were previously dispersed by the megafauna[55]. However, the opposite is observed in Chilean species. For example, *G. keule* populations (35–37° S) exhibit high genetic differentiation[56], whereas *J. chilensis* populations (33–34° S) show low genetic diversity and elevated inbreeding, despite some evidence of extensive past gene flow[40].

Second, smaller frugivores, livestock and humans may have partially replaced the seed dispersal services of the Pleistocene megafauna. However, several studies have shown that these substitutes have not fully compensated for the loss of megafaunal seed dispersers[17,56–59]. These findings have global implications, modern frugivores being unable to fully compensate for the unique seed dispersal services once provided by an extinct megafauna[21,60,61]. Moreover, research highlights that even extant large herbivores, such as modern-day proboscideans, provide limited functional redundancy compared to other seed dispersers[62,63].

Third, by integrating our palaeoecological and palaeoenvironmental data, which established a robust ecological link between *N. platensis* and megafaunal fruit plants in central Chile, with our model showing a

higher-than-expected extinction risk for these plants (Fig. 6), we can conclude that the loss of large frugivores may have seriously hindered the seed dispersal and recruitment of large-seeded plants.

Central Chile, recognized as a biodiversity hotspot[64], provides a crucial case study for understanding the ecological consequences of megafaunal extinction on LDSD in systems lacking effective surrogate dispersers. This knowledge is especially relevant considering the extensive defaunation that has occurred worldwide over the last 200 years. Our findings strongly support previous models that have suggested that megaherbivores played a pivotal role in maintaining plant diversity and shaping the distribution of megafaunal fruit plants[1,8,65]. Furthermore, these models emphasize how the loss of large dispersers can lead to significant declines in recruitment success, reductions in geographical range sizes and loss of genetic diversity. These effects will be even more pronounced if medium-sized frugivores are also removed from the system. While it may take more than 25,000 years for large-seeded plants to become extinct following the extinction of their dispersers[11], our results provide clear evidence of a heightened risk of extinction for plants that lack effective dispersers. This underscores the critical role that large frugivores played in shaping plant communities and highlights the far-reaching implications of their disappearance.

In conclusion, our study provides the smoking gun, in the form of robust fossil evidence, that strongly supports the JMNA hypothesis. We also highlight that the loss of key megafaunal seed dispersers probably triggers secondary extinctions, with significant long-term impacts on ecosystems. Understanding the lasting legacy of megafaunal extinctions on present-day ecosystems[61] is one of the remaining unknowns regarding extant biodiversity patterns[66]. Our findings thus provide valuable insights for conservation efforts, helping to predict the extent of functional changes that modern ecosystems may face under ongoing and future anthropogenic pressures[67].

Methods

A multiproxy approach, involving stable isotope and dental microwear analyses, and the analysis of microfossils from dental calculus, was carried out on 96 *N. platensis* molars. This investigation integrated newly collected samples with previously studied materials[31–33]. The samples selected for this study originated from 40 different sites, spanning latitudes between 31 and 36° S (north-central region, Mediterranean climate) and between 38 and 42° S (south-central region, temperate climate). The timeframe of these specimens spanned approximately 30,000 to 12,000 cal yr BP[32]. A comprehensive list of samples and their associated analyses can be found in the Source Data (Figs. 1–6). Not all molars were suitable for the multiproxy analysis, some exhibiting significant taphonomic and diagenetic alterations that precluded them from being analysed.

Ecological niche and environmental patterns

The stable isotope analyses shed light on the diet, trophic level and environmental and climatic variability of the studied specimens. To estimate the dietary composition and ecological niche of *N. platensis*, we performed stable carbon isotope analysis ($\delta^{13}C_{bioapatite}$) (Supplementary Table 1) on 96 molars from 40 sites (Fig. 1). We calculated the proportion of C_3 and C_4 plants in the diet using the Phillips (2012)[68] equations, as follows: (1) $\delta^{13}C_{mix} = \delta^{13}C_1 f_1 + \delta^{13}C_2 f_2$ and (2) $1 = f_1 + f_2$, where $\delta^{13}C_{mix}$ denotes the mean $\delta^{13}C$ values of C_3 (C_1 = −12 or −22‰) and C_4 (C_2 = +2 or −8‰) plants, corrected for a +15‰ bioapatite enrichment factor, with f_1 and f_2 representing the proportions of C_3 and C_4 plant consumption, respectively. We used these values to calculate the ecological niche breadth as $B = 1/\sum pi^2$, where pi is the proportion of each resource in the diet. This index was standardized from 0 (specialist diet) to 1 (generalist diet). To determine the types of forests where *N. platensis* foraged (as indicated by the estimated consumed plants) (Fig. 2), we analysed the stable isotopes in modern vegetation from Fray Jorge National Park (30° S) and Tagua Tagua (34° S) in north-central Chile, and from Tantauco Park (43° S) in south-central Chile (Fig. 1 and Supplementary Table 1). Differentiating the forest types was crucial because fleshy fruits are primarily found in the Mediterranean areas of central Chile. Analysing modern plants helped us determine whether the isotopic signals indicated the past presence of Mediterranean forests in areas once occupied by *N. platensis*. We evaluated whether the $\delta^{13}C$ values obtained from the *N. platensis* molars aligned with established stable isotope ranges that would account for the trophic fractionation and enrichment (for example, Domingo et al.[30]). Using this data, we built a baseline isotopic signature for the current vegetation that spanned ~1,500 km across various climate types in central Chile. These modern plant $\delta^{13}C$ values were then used to calibrate the $\delta^{13}C_{bioapatite}$ data from the *N. platensis* molars, approximating the probable isotopic signature of the vegetation during the time when the gomphotheres were extant.

We analysed 96 *N. platensis* molars to determine their meteoric water oxygen isotope composition ($\delta^{18}O_{meteoric\ water}$). This set included 24 new molars. To calibrate the $\delta^{18}O$ values from the *N. platensis* samples, we analysed eight molars from modern *P. puda* deer (Rivers District, southern Chile) (source data), chosen because this species inhabits a climate similar to that described for the north-central area during the Late Pleistocene. An estimated consumed plants value for the mammals was derived from a diet-to-tissue trophic discrimination study[8] and compared to modern vegetation. We applied the equation $\varepsilon^* = 2.4 + 0.034$ (bm) (bm, body mass; ε^*, $\delta^{13}C$ diet–bioapatite enrichment) to determine the enrichment between bioapatite and the diet of *P. puda* ($\varepsilon^*_{diet\text{-}bioapatite}$). Using an $\varepsilon^*_{diet\text{-}bioapatite}$ value of 12‰ for *P. puda* (body mass 9.6 kg), we improved the reliability of $\delta^{13}C_{bioapatite}$ comparisons across mammals with different body masses[2]. We used an enrichment of 14.1‰ ($\varepsilon^*_{diet\text{-}bioapatite}$) for gomphotheres[1,2]. Acevedo et al.[3], however, used an enrichment value of 15‰ ($\varepsilon^*_{diet\text{-}bioapatite}$) based on an estimated body mass of 6,000 kg. Given the variability in enrichment values, we employed a multiproxy approach and accounted for the isotopic proxy temporal resolution to reduce uncertainty.

We estimated the $\delta^{18}O_{meteoric\ water}$ ingested by gomphotheres from their enamel $\delta^{18}O_{PO4}$ values using a linear regression based on modern elephants, their closest living relatives. To carry out this analysis, we assumed no significant differences in the water-to-phosphate oxygen isotope fractionation factor between extinct gomphotheres and extant elephants. The equation used to obtain the $\delta^{18}O_{meteoric\ water}$ (Vienna standard mean ocean water (VSMOW)) value for the gomphotheres was $\delta^{18}O_{meteoric\ water\ (VSMOW)} = (\delta^{18}O_{PO4\ (VSMOW)} - 23.3)/0.94$. For the deer species *P. puda*, we used the equation for current ruminants of $\delta^{18}O_{meteoric\ water\ (VSMOW)} = (\delta^{18}O_{PO4\ (VSMOW)} - 25.53)/1.13$ (ref. 69).

We used $\delta^{13}C_{atmCO2} = -6.5‰$ as being consistent with the Late Pleistocene atmospheric CO_2 (ref. 5). The modern vegetation stable isotope data were corrected to account for the current $^{13}C_{atmCO2}$ composition of −8‰ (ref. 7). Powdered enamel samples (3.5–9.5 mg) were chemically analysed at the Biomolecular Laboratory of the Institut Català de Paleoecologia Humana i Evolució Social (IPHES), following the modified protocols of Koch et al.[70] and Tornero et al.[71]. Pretreated powders were analysed using a Thermo Kiel III device interfaced with a MAT Finnagan 253 at the Scientific and Technological Centers of the University of Barcelona (CCiTUB), Spain. Measurement accuracy was ensured using two internal laboratory calcium carbonate standards (RC-1 and CECC), normalized to international standards NBS18 and NBS19. Sixteen RC-1 and CECC samples were measured, with RC-1 expected at +2.83‰ $\delta^{13}C$ and CECC at −20.78‰ $\delta^{13}C$. The mean analytical precision was +0.01‰ for both standards. The stable isotope values followed the δ-notation $\delta^H X_{sample} = [(R_{sample} - R_{standard})/R_{standard}] \times 1{,}000$, where X is the element, H is the mass of the rare heavy isotope and R is the ratio ($^{13}C/^{12}C$ or $^{18}O/^{16}O$). The $\delta^{13}C$ and $\delta^{18}O$ values were expressed using the Vienna Pee Dee Belemnite (VPDB) standard, with the $\delta^{18}O$ also converted to the VSMOW scale using the formula $\delta^{18}O_{SMOW} = (1.0309 \times \delta^{18}O_{VPDB}) + 30.909$.

Palaeontological evidence for the anachronism hypothesis

Dental calculus, a mineralized form of bacterial plaque, contains particles that provide direct evidence of ancient diets and palaeoenvironmental conditions[58]. This proxy captures long-term dietary signals, spanning months to years[59]. We reanalysed molars from a previous study[26], triplicating the sampling points per molar and including additional molars for analysis. We used a multiproxy microfossil analysis[32,33] to infer the composition of the gomphothere diet and to comprehensively evaluate its frugivory. All recovered microfossils—phytoliths, starch granules, raphids and sclereids—were counted and recorded. New dental calculus deposits were also extracted from these new molar lophs (Supplementary Table 5). This study included 32 new samples, 27 exhibiting microfossil evidence of fruit consumption, such as starch, sclereids and raphids (Supplementary Table 4). Our methodology followed protocols from previous studies[32,33]. The analysed material is stored at the Universidad Austral de Chile (UACh) and Museo Nacional de Historia Natural (MNHN) in Chile.

Dental microwear analysis examines the enamel surfaces of teeth to infer the feeding habits in the last week of an animal's life[60]. We analysed a total of 21 molars (Supplementary Table 6) following the protocols of Solounias and Semprebon[44] and Semprebon et al.[72]. The enamel microwear was examined using a Zeiss Stemi 2000C stereomicroscope at 35× magnification on high-resolution epoxy casts. We included molars M1/m1, M2/m2 and M3/m3, as outlined by Xafis et al.[73]. Each molar's occlusal surface was cleaned first with acetone, followed by 96% ethanol. We then created high-resolution silicone mounds (Heraeus Kulzer, PROVIL novo Vinylpolysiloxane, Light C.D. 2 regular set) and produced transparent casts using clear epoxy resin (C.T.S. Spain, EPO 150 + K151). A 0.16-mm^2 ocular reticle was used to quantify the microwear features, including the number of pits (round scars), scratches (elongated, parallel-side scars), gouges (irregular and much larger and deeper than large pits), puncture pits (deep, crater-like pits with low refractivity), cross scratches (perpendicular to most scratches) and the SWS. An SWS score of 0 was given when only fine scratches were present, 1 when there was a mixture of fine and coarse scratches, 2 when coarse scratches were predominantly present, 3 when there was a mixture of coarse and hyper-coarse scratches and 4 when the surface also had hyper-coarse scratches. The mean numbers of scratches and pits were used to categorize the dietary habits as browsing (that is, eating woody and non-woody dicotyledonous plants), grazing (that is, eating grass) or mixed feeding by comparing the data to the known dietary habits of extant ungulates, including the three living elephant species[43,44].

Tree extinction risk modelling

Tree species database. We extracted a list of 20,557 tree species from the NeoTropTree (NTT) database[63]. The NTT database compiles inventories specific to a single vegetation type within a defined area of 5 km across Meso, Central and South America. These inventories include records of trees and tree-like species, defined as freestanding plants with stems that can exceed 3 m in height (see www.neotroptree.info for details). We then extracted georeferenced occurrences for these species to create a dataset of their distribution ranges.

Extinction risk categories. We extracted extinction risk categories for already classified species from the IUCN Red List's API66 through the redlist v.0.7 package. We obtained the IUCN extinction risk categories (that is, Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN) and Critically Endangered (CE)) for 9,584 tree species.

To determine the conservation status of uncategorized tree species in the NTT database, we implemented a fully connected neural network model (nn-class) using the IUCNN R package, as presented by Zizka et al.[74]. The model, trained with species already assessed by the IUCN, uses species distribution features as inputs. For training, we provided 1,048,576 georeferenced occurrences for 20,557 species extracted from the NTT, along with the extinction risk categories of 9,584 known species as labels. The IUCNN package generates 45 quantitative and categorical features at the species level, including species range characteristics, the human impact within the species range, climatic conditions and biome occurrences, using publicly available environmental data for georeferenced points[74].

We optimized the neural network within a TensorFlow framework, using a subset of species with known extinction risk categories. The nn-class model predicts the extinction category with the highest probability[74]. The probabilities are then used to compute cross-entropy loss, averaged across all training instances, and minimized during the optimization. We adopted a five-fold cross-validation, splitting the data into 80% for training and 20% for validation, to assess the model's predictive performance. Following initial exploratory trials, we set the neural network's architecture to three hidden layers with 50, 30 and 10 nodes, respectively. The trained model, with an overall accuracy of 0.51, was used to predict the extinction risk of the 10,973 unlabelled species. Confusion matrices showed that classification errors between the five conservation labels occurred mainly between closely related risk categories, especially between CR, EN and VU. To avoid the propagation of classification errors and their potential influence on the conclusions, we grouped species into two broad categories: Under risk, which included VU, EN and CR, and Low concern, which included species listed as LC and NT. We combined these predictions with the available risk label assessments, grouped in the same way, to summarize the estimated extinction risk for the entire dataset.

Tree species according to fruit type. We compared the extinction of tree species in central Chile where the megafauna was extinct with other regions of the neotropics still inhabited by fauna capable of dispersing megafaunal fruits (Fig. 6). Using level 3 of the World Geographical Scheme for Recording Plant Distribution (formerly known as the Taxonomic Databases Working Group[45]) we defined four botanical countries: Chile central, Brazil south, Brazil west-central and Mexico southeast (Supplementary Data). We extracted a list of 6,067 tree species (4,120 species with IUCN-assigned categories and 1,947 species with predicted extinction risk classifications) from these botanical countries and grouped those bearing fleshy fruits into two categories: species with megafaunal seed dispersal traits and those without. This classification involved several steps. First, we identified plant species with fleshy fruits or fleshy arils that vertebrates could consume. Next, we categorized these species based on their fruit characteristics, determining whether they exhibited a megafaunal or non-megafaunal seed dispersal syndrome. We defined a fruit as megafaunal if: (1) it fell within a size range of 4–10 cm in diameter and contained up to five large seeds; or (2) it exceeded 10 cm in diameter and contained numerous small or a few large seeds[8]. We collected fruit data from herbarium measurements and the existing bibliography. In total, we identified 3,212 tree species with fleshy fruits across the four botanical countries, 286 of which had fruits with megafaunal characteristics (see the source data for Fig. 6 for details).

Statistical significance. To assess the significance of the observed proportions of extinction risk by fruit type across the botanical countries (Fig. 6), we compared our results against an expected outcome derived from a null model. This model randomly assigned species and their conservation statuses to the four botanical countries, drawing from the overall species pool in the community matrix. Species were allocated without replacement, ensuring each was assigned only once. We generated 1,000 simulated datasets for each botanical country to construct a null distribution of conservation status proportions. Observed proportions falling within the lowest or highest 5% of the null distribution were considered significantly different random expectations (two-tailed test set at $\alpha = 0.1$). All statistical analyses were conducted in the R statistical environment[75]. A repository with the codes can be found at the link specified in the Code availability section.

Reporting summary
Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability
Datasets are provided at https://github.com/ricardosegovia/Gonzalez-Guarda_etal_2025. Source data are provided with this paper.

Code availability
Code is provided at https://github.com/ricardosegovia/Gonzalez-Guarda_etal_2025.

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Author contributions

E.G.-G, project leader, led the manuscript writing, performed the analyses and supervised all aspects of the paper. A.P.L. co-authored a significant portion of the manuscript and categorized the species in the tree species database as megafaunal or non-megafaunal fruit plants. R.A.S. co-authored a significant portion of the manuscript and performed the tree risk extinction modelling analyses. A.P.-P. co-authored parts of the Methods and Results sections and conducted the analysis of the dental calculus samples. I.R.-P. co-authored parts of the Methods and Results sections and conducted the analysis of the dental microwear samples. L.A. co-authored parts of the Methods and Results sections and conducted the analysis of the ecological niche of *N. platensis*. C.T. contributed to the laboratory work, specifically regarding the stable isotope analyses. R.L. acquired the dental calculus samples from the *N. platensis* molars. F.R. assisted in writing and revising the manuscript and analysed the dental microwear samples. C.L. significantly contributed to the supervision, writing and editing of the manuscript, and provided additional funding and analyses.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Erwin González-Guarda or Claudio Latorre.

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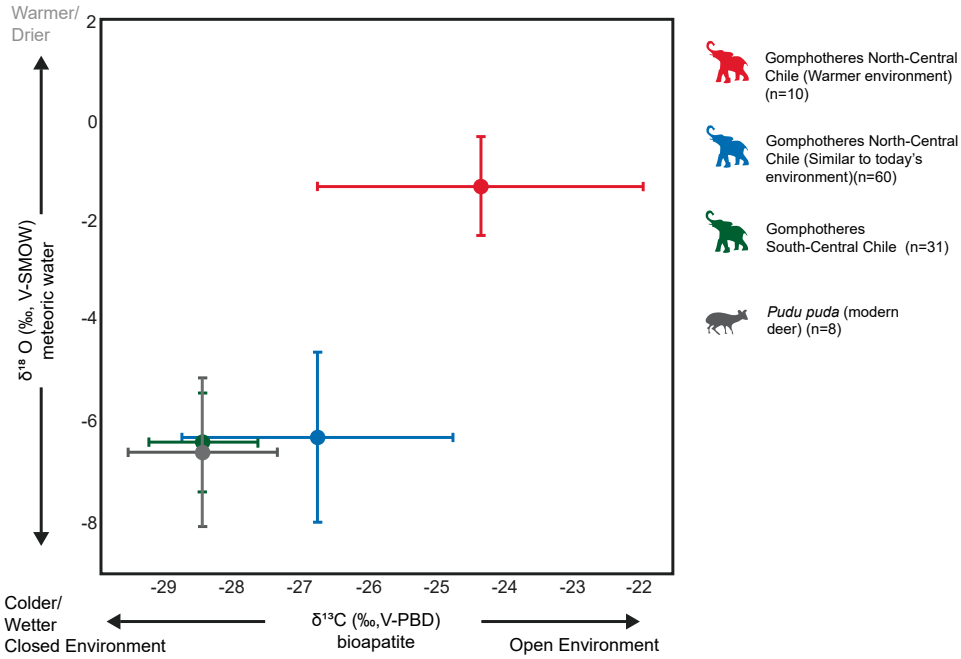

Extended Data Fig. 1 | Isotopic comparison between gomphotheres and modern fauna in central Chile. Graphical results of isotopic analyses derived from multiple sources showing the mean values of $\delta^{13}C_{bioapatite}$ (‰, VPDB) and $\delta^{18}O_{meteoric\ water}$ (‰, SMOW) for *Notiomastodon platensis* and *Pudu puda*.

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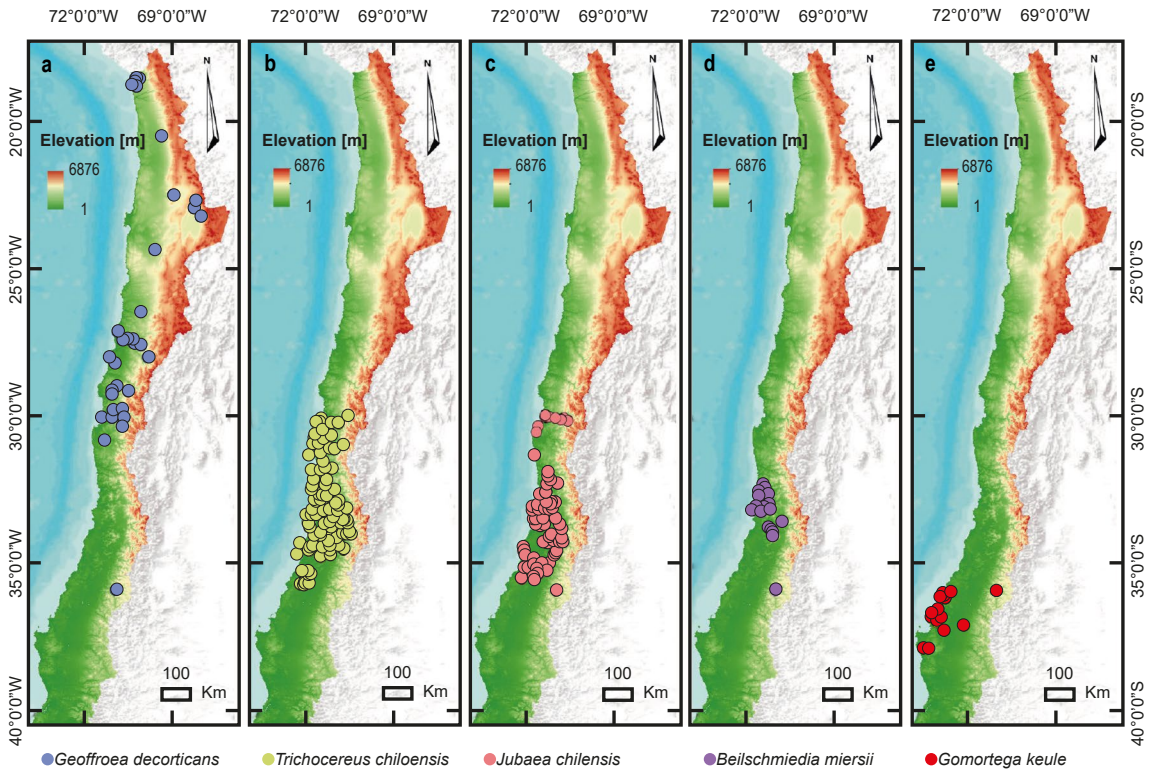

Extended Data Fig. 2 | Distribution of plants with fleshy fruits in central Chile. The geographical distribution of the main species of fleshy fruits that we analyzed in central Chile is observed. a, *Geoffroea decorticans*. b, *Trichocereus chilensis*. c, *Jubaea chilensis*. d, *Beilschmiedia miersii*. e, *Gomortega keule*.

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Study description	Samples of plants from relatively closed environments were analyzed, to determine the types of forests where N. platensis foraged (as indicated by Estimated Consumed Plants; ECP). It is important to note that the analysis of plant samples is only referential and complementary to the finding of frugivory. However, the results agree with the ecological niche model carried out, and with the stable isotope ranges that have been established to characterize environments from mammals.
Research sample	We analyzed stable isotopes in modern vegetation at Fray Jorge National Park (30ºS) and Tagua Tagua (34ºS) in north-central Chile, and in Tantauco Park (43ºS) in south-central Chile. 237 plants were analyzed.
Sampling strategy	Descriptive statistics, which uses indices such as the mean and median to summarize and associate data. Phytosociological sampling was used. With this method we can obtain the dominant species through coverage. First you have to find a homogeneous area and identify the existing species. Subsequently, the dominant species must be identified. Furthermore, in each plot a geomorphological or climatic characteristic must be identified that allows the plots to be grouped.
Data collection	The collection of the samples was carried out by researcher Erwin González, who used pruning shears to take the samples.
Timing and spatial scale	The sampling date was chosen because it is the period of the year with the least amount of rain; which allowed expeditious access to sampling locations. 5 large sampling areas were selected, under different climates, along 1500 km.
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Reproducibility	The plants that were used for the study are not in danger of extinction, therefore, they can be studied again according to the same method used.
Randomization	Samples were obtained in controlled environments and climates (wetter and more arid). In addition, an effort was made to take samples in closed canopy areas.

Blinding	One of the methodological biases is related to the fact that the plants analyzed in this study live in specific environments and climates. Therefore, we might expect stable isotope values typical for those types of environments.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	Throughout the 1500 km of the sampling area, different weather patterns were observed. Environmental conditions range from semi-arid environments to temperate rainforests.
Location	Fray Jorge National Park (Hygrophilous forest) (30º 30¨¨S, 71º 35′ W) (wetter area). 1000 mm/yr. 13.7 ºC Fray Jorge National Park (Semi-arid scrub) (30º 30′ S, 71º 35′ W) (arid area) 147 mm/yr. 13.7 ºC. Tagua Tagua (Sclerophyllous forest) (34° 33' S, 71º 9' W) (wetter area). 800 mm/yr. 15 ºC. Tagua Tagua (Sclerophyllous forest) (34º 30' S, 71º 1' W) (more arid area). 800 mm/yr. 15 ºC. Tantauco Park (Temperate Rain forest) (43° 12′ S, 74º 11′ W) (wetter area). 3000 mm/yr. 10 ºC
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